exposure to 20E for 6h. CHR3 mRNA started increasing at 3h and rose to the maximum levels within 6h; after continuous exposure to 20E for 12h, the mRNA levels started to decrease.

We used cDNA probes for these four receptors to study the molecular mode of action of RH-5992. CfECR, CHR75 and CHR3 mRNAs were induced in the midgut, fat body and epidermis of RH-5992-fed larvae. The induction pattern of these three receptors in the midgut, epidermis and fat body of RH-5992-fed larvae was exactly the same as that described above for 20E induction in CF-203 cells. As with 20E, RH-5992 also did not induce CfUSP mRNA. These results showed that RH-5992 acts in a similar way to 20E in causing gene expression events that occur in the presence of 20E. However, the end result of 20E increase was a successful molt, whereas increasing RH-5992 resulted in an incomplete molt. One of the possible reasons for this difference is the persistence of RH-5992 in the tissues. After initial positive regulation, 20E titres decrease, allowing the expression of genes that require the absence of 20E for their expression (eg dopadecarboxylase and larval cuticular proteins). On the other hand, RH-5992 titre does not decrease but persists in the cells and thus does not allow the expression of these genes, resulting in an incomplete molt.

RH-5992 caused the above effects on the *C fumiferana* larvae only if the larvae ingested the compound prior to the appearance of the endogenous 20E peak. If the compound was administered after the endogenous peak of 20E, the larvae molted normally into the next stage. However, the compound manifested its typical effects at the early stage of the next stadium. Quantification of CHR3 mRNA levels in the midgut, fat body and epidermis of the larvae treated with RH-5992 on each day of the last larval stage showed that the tissues were not becoming refractive to this compound after they were exposed to endogenous 20E. RH-5992 induced CHR3 mRNA throughout the 6th larval stage. These studies revealed two important properties of RH-5992: first, it can only initiate the molting process and cannot restart the molting process after endogenous 20E has initiated it and second, it is stable in the insect and can be carried over to the next larval stage where it can induce precocious molting.

ACKNOWLEDGEMENTS

The study was supported by Canadian Forest Service/ Natural Sciences and Engineering Research Council Research Partnership program award 0192158, with the Rohm and Haas Company.

REFERENCES

1 Wing KD, RH-5849 a non-steroidal ecdysone agonist: Effects on a Drosophila cell line. *Science* (*Washington*) **241**:467–469 (1998).
2 Wing DD, Slawecki RA and Carlson GR, RH-5849, a nonsteroidal ecdysone agonist: effects on larval Lepidoptera. *Science* (*Washington*) **241**:470–472 (1988).
3 Retnakaran A, Macdonald A, Tomkins W, Davis C, Brownright AJ and Palli SR, Ultrastructural effects of a non-steroidal ecdysone agonist, RH-5992, on the sixth-instar larva of the spruce budworm, *Choristoneura fumiferana*. *J Insect Physiol* **43**:55–68 (1996).
4 Kothapalli R, Palli SR, Ladd TR, Sohi SS, Cress D, Dhadialla TS and Retnakaran A, Cloning and developmental expression of the ecdysone receptor from spruce budworm, *Choristoneura fumiferana*. *Dev Genet* **17**:319–330 (1995).
5 Perera SC, Palli SR, Ladd TR, Krell PJ and Retnakaran A, The ultraspiracle of the spruce budworm, *Choristoneura fumiferana:* cloning cDNA and developmental expression of mRNA. *Dev Genet* **22**:169–179 (1998).
6 Palli SR, Ladd TR, Sohi SS, Cook BJ and Retnakaran A, Cloning and developmental expression of *Choristoneura* hormone receptor 3, an ecdysone-inducible gene and a member of the steroid hormone receptor superfamily. *Insect Biochem Mol Biol* **26**:485–489 (1996).
7 Palli SR, Ladd TR, Ricci AR, Sohi SS and Retnakaran A, Cloning and developmental expression of *Choristoneura* hormone receptor 75:A homologue of the *Drosophila* E75A gene. *Dev Genet* **20**:36–46 (1997).

Chemical catalysis of the isomerisation of peroxidising herbicidal thiadiazolidines

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Abstract: Model reactions with various -SH, -OH and -NH nucleophiles in an aprotic solvent were used to characterise the nature of the enzymatic conversion of thiadiazolidines to triazolidines. This conversion results in greater inhibition of protoporphrinogen oxidase (protox). It is inferred that GST-isoforms probably promote formation of one of the intermediates.

Keywords: catalysed bioconversion; protox inhibitors; thiadiazolidines; triazolidines; GST-isoforms

5-Arylimino-3,4-tetramethylene-1,3,4-thiadiazolidin-2-ones (thiadiazolidines **I**. Fig 1) are peroxidising bleaching herbicides that act by interrupting chlorophyll biosynthesis *via* inhibiting the activity of the enzyme protoporphyrinogen oxidase (protox, EC 1.3.3.4).[1]

Bioconversion of **I** into isomeric 4-aryl-1,2-tetramethylene-1,2,4-triazolidin-5-thiones (triazolidines **II**, Fig 1) results in bioactivation, ie stronger inhibitors

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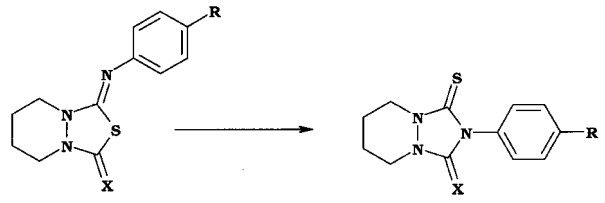
(*Received 27 August 1998; accepted 16 February 1999*)



I **II**

Ia (BW 78) and **IIa** (BW 85) X = O, R = Br
Ib (BW 83) and **IIb** (BW 91) X = O, R = 4-Cl-benzyloxy
Ic (BW 114) and **IIc** (BW 121) X = O, R = Br

Figure 1. Chemical structures of the compounds investigated.

of protox.[2,3] In plants this isomerisation requires the catalytic action of an isoenzyme of glutathione *S*-transferases (GSTs, EC 2.5.1.18, important enzymes in detoxification reactions of xenobiotics).[2–4] Isomerase activity of a maize GST isoform was dependent on reagents containing a thiol group such as dithiothreitol, thioglycolate or reduced glutatione (GSH)[5] as cofactors in the isomerisation. Although non-enzymatic interconversion of a structurally related tetrahydroisophthalimide to a tetrahydrophthalimide in water has been observed,[6] no such transformations of thiadiazolidines and triazolidines were detected in buffer solution.[1,3]

The objective of this study was to characterise the nature of enzymatic conversion of thiadiazolidines **I** to triazolidines **II** by carrying out model reactions in an aprotic solvent in the presence of various -SH, -OH, and -NH nucleophiles. The use of aprotic solvent instead of water was required to mimic the environment of the hydrophobic pocket of GST. Isomerisation reactions were followed as described previously.[7]

We found that, with the exception of pyrrolidine, alkyl- and aryl-SH, -OH, and -NH compounds alone were unable to trigger detectable isomerisation (Table 1). Tertiary amines, such as the non-nucleophilic base 1,8-diazabicyclo [5.4.0]undec-7-ene. (DBU),[8] 4-dimethylaminopyridine (DMAP) and 2,6-lutidine also exhibited little or no isomerisation catalytic activity (Table 1). Surprisingly, mixtures of DBU with various -SH, -OH, and -NH nucleophiles were highly catalytic (Table 1). Using -OH compounds, lower isomerisation rates were detected as compared with thiols, as indicated by higher half-life values (Table 1).

In the amine nucleophile series we used imidazole, pyrrolidine, and indole which are backbones of histidine, proline and tryptophane amino acids, important building blocks of several GST enzymes.[9] These amines showed a wide range of reactivity. Pyrrolidine itself was able to induce a rapid isomerisation with all substrates (Table 1).

The rearrangement mechanism probably involves several intermediates. Under our experimental conditions these intermediates seemed to be unstable, because only the starting material and the end-product were detectable during the whole isomerisation process. The catalytic role of the specific GST-II isoform in the isomerisation[6] is probably the promotion of formation of one of the intermediates.

Table 1. Influence of nucleophiles and bases on isomerisation of thiadiazolidines

	Time required for 50% isomerisation (h)		
Cofactor	*BW78*	*BW83*	*BW114*
Nucleophile[a]			
Butane-l-thiol	0.06	0.52	0.05
Thiophenol	0.25 (31.30[b])	1.33	0.31
2-Mercapto-ethanol	0.07	–	–
Cysteine	5.60	–	–
GSH	Not studied[c]	–	–
n-Butanol	38.60	–	–
Phenol	21.60	25.17	52.20
Pyrrolidine[d]	0.07	0.48	1.06
Imidazole	12.30	–	–
Indole	46.50	–	–
Base[e]			
DBU	46.90	–	–
DMAP	44.70[f]	–	–
2,6-Lutidine	No isomerisation	–	–

[a] Isomerisation with nucleophiles in the presence of DBU.
[b] Isomerisation rate of BW78 with thiophenol in the presence of DMAP.
[c] Not studied because of the insufficient solubility of GSH in acetonitrile.
[d] Isomerisation rate with pyrrolidine alone.
[e] Isomerisation of BW78 only with respective base.
[f] Time required for 10% isomerisation.

REFERENCES

1 Sato Y, Hoshi T, Iida T, Nicolaus B, Wakabayashi K and Böger P, Isomerization and peroxidizing phytotoxicity of thiadiazolidine herbicides. *Z Naturforsch* **49c**:49–56 (1994).

2 Seeno S, Iida T, Shouda K, Sato Y, Nicolaus B, Böger P and Wakabayashi K, Enzyme-modified phytotoxic structure of thiadiazolidine compounds. *Z Naturforsch* **51c**:518–526 (1996).

3 Nicolaus B, Sato Y, Wakabayashi K and Böger P, Isomerization of peroxidizing thiadiazolidine herbicides is catalyzed by glutathione *S*-transferase. *Z Naturforsch* **51c**:342–354 (1996).

4 Rossini L, Jepson I, Greenland AJ and Sari Gorla M, Characterization of glutathione *S*-transferase isoforms in three maize inbred lines exhibiting differential sensitivity to alachlor. *Plant Physiol* **112**:1595–1600 (1996).

5 Iida T, Seeno S, Sato Y, Nicolaus B, Wakabayashi K and Böger P, Isomerization and peroxidizing phytotoxicity of thiadiazolidine-thione compounds. *Z Naturforsch* **50c**:186–192 (1995).

6 Sato Y, Böger P and Wakabayashi K, The enzymatic activation of a peroxidizing cyclic isoimide: a new function of glutathione *S*-transferase and glutathione. *Nihon Noyaku Gakkaishi (J Pestic Sci)* **22**:33–36 (1997).

7 Jablonkai I, Kömives T, Böger P, Sato Y and Wakabayashi K, Chemical catalysis of the isomerization of a peroxidizing herbicidal thiadiazolidine. *Proc Brighton Crop Prot Conf–Weeds* vol 2, pp 771–776 (1997).

8 Barton TJ, Martz MD and Zika RG, Facile bridge expulsion of sulfur heterocycles. *J Org Chem* **37**:552–554 (1972).

9 Jepson I, Lay VJ, Holt DC, Bright SWJ and Greenland AJ, Cloning and characterization of maize herbicide safener-induced cDNAs encoding subunits of glutathione *S*-transferase isoforms I, II, and IV. *Plant Mol Biol* **26**:1855–1866 (1994).